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OBSERVATIONS ON *CHILOMASTIX INTESTINALIS* KUCZINSKI

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INTRODUCTION

Species of Chilomastix have been reported from a number of vertebrate hosts, viz., tadpole, fish, rat, goat, rabbit, guinea-pig and man. While species in this genus are based largely on characters such as size, cytostome, and differences in cysts, the species all possess morphological features which are so strikingly in common that they easily fall to the one genus. Among these common structural characteristics are the pear-shaped bodies of many of the trophozoites, the presence of a lipped cytostome with the cytostomal flagellum, the three anterior flagella, and the pyriform cyst.

It is worthy of note that the generic name Chilomastix has been adopted only recently to designate these species. This was largely brought about by a thorough revision by Chalmers and Pekkola (1917) of the work done within a period of a little over fifty years which resulted in reducing the number of tenable species from sixteen to seven, the other nine now standing only as synonyms of *Chilomastix mesnili* (= *Chilomastix davainei* Moquin-Tandon), the parasite of man. The name Chilomastix should stand as has been urged by Alexeieff (1912) in preference to Macrostoma as this name was originally used by Latreille (1825) for a mollusc, and also to Tetramitus, because of the absence of a trailing flagellum in Chilomastix.

The first record of species belonging to this genus is probably that of Davaine (1854) who reported from the motions of cholera patients during an epidemic in Paris, a parasitic flagellate under the name of *Cercomonas hominis* var. A. This species Moquin-Tandon named *C. davainei,* and Wenyon later described it as *Macrostoma mesnili.* Its correct name has been determined by Kofoid (1920) to be *Chilomastix davainei* (Moquin-Tandon). Alexeieff (1910) was the first to describe with considerable detail a species of this genus, calling it *Tetramitus caulleryi,* but in 1910 and 1912 he referred it to a new genus *Chilomastix.* This species is found as a lumen-dwelling parasite of the intestine of the tadpoles and axolotl.

Chilomastix intestinalis, the subject of this study, was found in the cecum of the guinea-pig and described very briefly by Kuczinski (1914) in his work of trichomonad flagellates. Being a parasite of an extensively used laboratory animal and related to the human parasite, it is thus not without general interest.

These investigations were carried out at the instance of Professor Charles A. Kofoid for whose help and criticism I am deeply grateful. I also wish to express my appreciation of the kind assistance rendered me in the laboratory by Dr. O. Swezy, and to both for access to their then unpublished drawings of *Chilomastix davainei* (see Kofoid and Swezy, 1920).

MATERIAL AND TECNIQUE

Chilomastix intestinalis was detected in 11% of the guinea-pigs examined during the course of the present work, excluding those animals which were artificially infected. The living forms were studied by placing a small amount of the intestinal contents mixed with a little normal saline solution on a slide, covering it with a coverslip and sealing it with vaseline. Care was observed not to dilute it with too much liquid so that too rapid a movement by the organisms could be avoided, and thereby the actual motions of the body could be watched with ease.

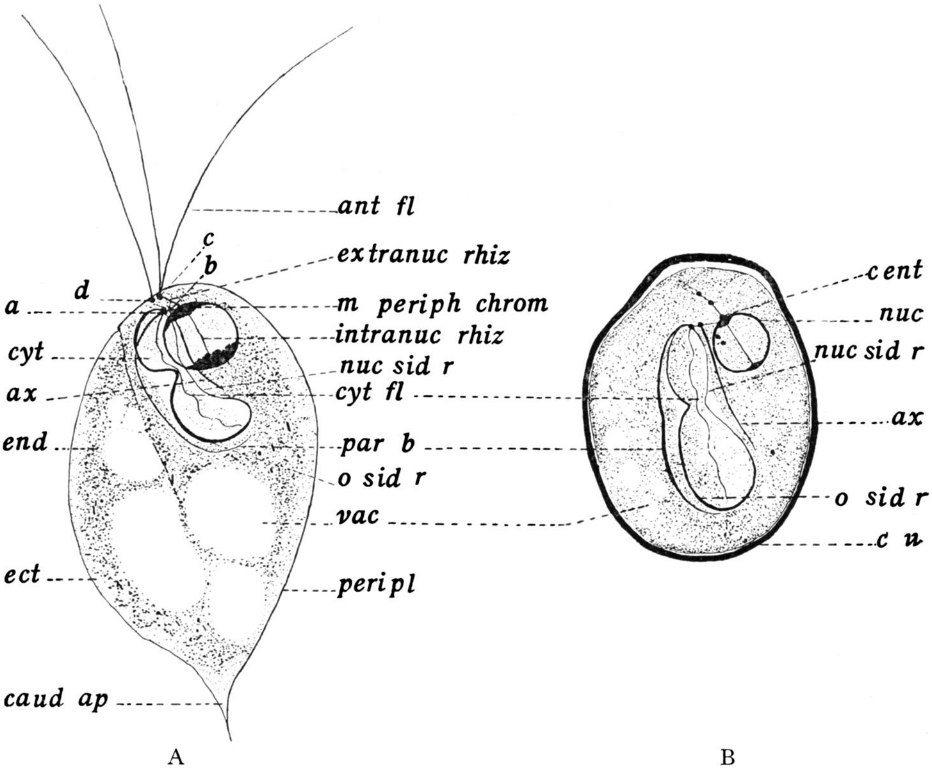
The Heidenhain iron-haematoxylin method of staining after fixation of the flagellates in Schaudinn's fluid was used in all cases for permanent mounts. The study of the cysts was facilitated by the employment of Kofoid's (1919) modified Donaldson's iodine-eosin stain. Chilomastix cysts readily became distinguishable in this mixture as pyriform bodies with the cytoplasm stained light pink and the cytostome standing out with clearness. With this stain also, a good definition of the flagella of the free forms is secured.

To determine the place of occurrence of the parasites the alimentary tract from the oesophageal opening of the stomach down to the rectum was taken out of the abdominal cavity and opened up with a pair of clean scissors at points about an inch apart, and material from each location was examined. This was done with two guinea-pigs which were found to be naturally harboring *C. intestinalis* and in two which were artifically infected.

From the results of the examinations it will be seen that, contrary to the findings of previous workers, this species is not exclusively a cecal parasite in the guinea-pig. In these hosts free and encysted stages of the flagellate were found beginning two inches above the ileo-cecal junction, though the infection is usually heaviest in the cecum. Extending from the cecum down to a distance of about 15 inches below it both stages of the parasite are encountered in fairly large numbers. It is only beyond this point where the fecal material begins to be less fluid in consistency and harder fecal scybala are formed that cysts only are to be found. No evidence of tissue-invasion has been detected from the study of sections taken from various portions of the gut.

MORPHOLOGY OF THE FREE STAGES

The free form has been watched many times continuously for considerable time so as to determine the normal course of movement. It has been seen that generally it travels not in a straight line but in a rather sinuous fashion with the anterior portion of the body undergoing a sort of spiral rotary motion. This is evidenced by the changing position of the cytostome which at one moment is on the left border of the organism, next on its right, to disappear as the ventral surface (i. e.



A, ordinary trophozoite of *Chilomastix intestinalis;* B, cyst. × 3840. *a, b, c, d,* blepharoplasts; *ant. fl.,* anterior flagellum; *ax,* axostyle; *c w,* cyst wall; *cent,* centrosome; *caud ap,* caudal appendage; *cyt,* cytostome; *cyt fl,* cytostomal flagellum; *extranuc rhiz,* extranuclear rhizoplast; *ect,* ectoplasm; *end,* endoplasm; *intranuc rhiz,* intraneuclear rhizoplast; *m perph chrom,* massed peripheral chromatin; *nuc,* nucleus; *nuc sid r,* nuclear siderophilous rim of the cytostome; *o sid r,* outer siderophilous rim of the cytostome; *par b,* parabasal body; *peripl,* periplast; *vac,* vacuole.

taking the surface where the cytostome is situated as ventral) is rotated dorsad. Sometimes this turning around of the anterior portion of the body with the posterior part following suite reveals a characteristic twist or spiral groove (Kofoid and Swezy, 1920) in some of these flagellates. This is apparent particularly in stained preparations in which there is seen in certain instances a spiral groove curving diagonally around the posterior part of the body (Fig. 5).

Metabolic movements of the body take place although not as marked as in trichomonad flagellates. A succession of diverse shapes may be seen in one individual such as the rounding out of a previously pointed posterior extremity (Figs. 1 and 2), or from a pear-shaped to a more rotund form. Irregular forms or even clubbed and elongated processes may thus be produced (Fig. 8). On the average the trophozoite is 12μ in length including the tail and 8μ in greatest diameter. Extremes in size have been encountered, as for instance, individuals measuring as long as 16.5μ and as short as 7.2μ (Fig. 1). Judging from the size of the latter it seems that it may have just emerged from the cyst. The nucleus is invisible in the fresh specimen, while the cytostome stands out with its prominent lips, with the posteriorly directed cytostomal flagellum undulating inside throughout its entire length.

Study of the stained specimens shows that there is rather definite ecto- and endoplasmic differentiation. The ectoplasm (*ect,* Fig. A) takes the stain lightly and is finely granular. The endoplasm (*end*) appears more heavily colored and its granulations coarser than those of the ectoplasm. The periplast (*peripl*), is the thin coating of the cell which is made up of differentiated ectoplasm and whose elasticity allows a certain degree of metabolic movements above described. Posteriorly it may be prolonged as a pointed caudal appendage which is free of the granular cytoplasmic material. The length of this process is variable, measuring on an average 4μ. Exceptionally long appendages (Fig. 4) may measure even 12μ. Tailless individuals with rounded posterior ends are encountered and they are not few (Figs. 2, 6). In the general run of cases the body takes on a variety of forms ranging from pear-shaped to those of oval or more rotund or of irregular contour. Vacuoles (*vac,* Figs. A, B) of various sizes are present and not infrequently they fill almost the entire endoplasmic area extending between the nucleus and that level of the body where it distinctly tapers down to form the caudal appendage. Bacterial masses form the common cell inclusions.

The nucleus (*nuc,* Fig. B) is situated close to the anterior margin of the body. While as a rule it is rounded in outline, oval forms are not rare. Overstained specimens show it to be a dense dark mass. However, if destaining is carried far enough, it becomes apparent that it consists of a heavy and firm nuclear wall of uniform thickness, and encrusted on the inner side, commonly at opposite poles, are the peripheral chromatin masses. Running between them a faintly staining line, the intranuclear rhizoplast (*intranuc rhiz,* Fig. A) can be seen on careful focusing. If only one peripheral chromatin block is present it is usually seen to lie on the anterior side of the nucleus (Fig. 3). Still another type of chromatin arrangement is that in which it is massed at the center of the nucleus as a karyosome (Fig. 10) ; or the chromatin may be broken up into small particles which lie scattered in the nucleus (Fig. 5). I was able to count as many as sixteen of these chromosome-like (?) bodies in one nucleus.

The cytostome and the structures immediately adjoining it have been in the past the subject of diverse interpretations, for the reason that not all of the component parts were identified so as to allow for the complete analysis of their relations in the organism. Heretofore, in the characterization of the different members of the genus Chilomastix, there have been described as invariably present a mouth opening or cytostome provided with rather prominent lips. Chalmers and Pekkola described in *Chilomastix mesnili* distinct siderophilous thickenings along the margins of the lips. Kuczinski's figures of *C. intestinalis* are very confused and vague, giving no clear analysis of the cytostomal region. Kofoid and Swezy (1920) working on the same species have fully analyzed this region and have found that in addition to those lines which are in the cytostomal rim, there are two other lines each running along the basal portion of the lips. They attribute to these two lines a parabasal and an axostylar (parastyle) significance respectively. In working over *Chilomastix intestinalis* from the guinea-pig I have found the same structures and they will now be described in this species.

The cytostome (*cyt,* Fig. A) is a lipped groove running from a level about the upper margin of the nucleus caudally and laterally to near the middle of the body. It measures on the average 5μ in length. Its breadth is quite variable since either approximation or drawing apart of the lips naturally modifies it. When the opening is fully patent it attains a width of 3μ. It is usually directed obliquely posteriorly with its opening wholly looking from one surface of the body. The shape is characteristic, the opening being composed of two wide spaces connected together by a narrow passage (Fig. 1). Such a figure is brought about by the curving middleward of the two sides of the lip with their convexities very closely approximated in some instances (Fig. 3), thus constricting the opening of the cytostome. The lower space is usually the rounder of the two. Of the two margins that on the side next to the nucleus or the nuclear siderophilous rim (*nuc sid r,* Fig. B) generally appears less wavy and more lightly stained. They both originate from a common blepharoplast (*a,* Fig. A). From this blepharoplast there also arises the cytostomic flagellum (*cyt fl,* Figs. A, B) and a more or less heavily stained curved linear structure (*parab b*) which curves around the base of the outer lip. The latter organelle is the one considered by Kofoid as the homologue of the parabasal body as seen in other flagellates. At times it is quite elongated and follows the contour of the outer siderophilous rim (Fig. 2).

At the base of the lip proximal to the nucleus there runs a lightly staining line which has its origin from another blepharoplast (*b,* Fig. A). It curves around the base of this lip and seems to show in several instances a tendency to meet the parabasal body. This was called the parastyle and is supposed to correspond to the axostyle.

Besides the two blepharoplasts already referred to there are often encountered two others. One (*c,* Fig. A) gives a common origin to two anterior flagella, and another (*d*) gives rise to the third free flagellum. However, it happens not infrequently that the three anterior flagella take origin from a single blepharoplast (Fig. 2). In this instance there will therefore be only three blepharoplasts instead of the four which are of more common occurrence. Rarely there are three distinct blepharoplasts for the anterior flagella (Fig. 6). The blepharoplasts are connected with each other and the nucleus by means of the extranuclear rhizoplasts (*extranuc rhiz,* Fig. A).

ENCYSTED STAGES

Cysts of various dimensions have been encountered during the course of these examinations. In examining material from one host alone, multiple infection with more than one strain of the same parasite cannot be excluded. On the average the cyst measures 7.9μ along its long axis, and 7.2μ in its greatest transverse diameter. Very small cysts whose dimensions are about 5 by 3.8μ have been met with. On the other hand large ones measuring around 10.8 by 8.3μ also do occur.

The shape of the cyst is, as a rule, broadly pyriform, with one pole narrower than the other, with a slight, gradual constriction at about the junction of the anterior and posterior two-thirds. Spheroidal, oval and ellipsoidal form of cysts are not uncommon. There is a rather thick wall (*c w,* Fig. B) which is separated from the cyst contents by a space. This space is particularly evident at the anterior and narrower end of the cyst. In pyriform cysts the wall is uniformly thicker at the top than elsewhere. Within the cyst chromatoidal inclusions are frequently seen scattered throughout, sometimes as darkly staining ellipsoidal bodies (Fig. 7) and at other times as narrow chromatinic splinters (Fig. 11).

Diverse types of nuclear organization have been encountered in these cysts; some of them in all probability represent division phases.

There may be a nucleus with apparently no peripheral chromatin but with a distinct subcentral karyosome (Figs. 10, 12). This chromatin mass may appear divided (Fig. 14) into two; in which case it is brought about by the division of the centrosome, the daughter centrosomes traveling apart, drawing out a paradesmose between them. Nuclei with no central karyosome but with the peripheral chromatin encrusted at opposite poles are the ones commonly seen (*m periph chrom,* Fig. A; Fig. 11). The remarkable feature in most of these cysts, in addition to its shape, is the presence of the cytostomal or oral loop (*cyt,* Fig. A). The parabasal body (*Par b,* Figs. A, B) appears particularly more distinct in the encysted than in the free stages. The axostylar structure (*ax*) can be seen especially with well-oriented and with carefully differentiated cysts.

Evidence of mitosis, both in the trophozoite and in encysted stages has been searched for. None has been seen in the free forms while an unmistakable telophase was encountered in one cyst (Fig. 14). The chromatin material in the nucleus appears to be divided, with the centrosomes at corresponding poles outside the nucleus and connected with each other by a paradesmose. The mouth parts, the parabasal and axostylar structures are duplicated, a new set apparently arising anew from distinct blepharoplasts.

As in other flagellates, the well-regulated action of the different kinds of flagella may be attributed to the existence of an integrated, extranuclear organelle, the neuromotor system. Its component parts are the blepharoplasts which are connected to the nucleus by means of rhizoplasts, the parabasal and axostyle, the cytostomal and the free anterior flagella. It is remarkable that the parabasal and axostylar structures are both associated around the region of the cytostome, an actively movable part of the organism. They certainly in a measure contribute to the motor functioning of the oral aperture, interpreting the parabasal body as a kind of reservoir for motor activity of the neuromotor apparatus, and the axostyle as a modified intracytoplasmic flagellum.

Chilomastix intestinalis as a distinct species

Da Fonesca (1916), after Kuczinski (1914) first gave a brief account on this parasite, described it more fully as a separate species. Their published observations, however, seemed not to have convinced Chalmers and Pekkola (1917), and they regard it as similar to and the same species as the Chilomastix of man. In the light of the present work certain striking differences are noticed between the two forms, so that one is forced to the conclusion that the Chilomastix of the guinea-pig should be considered as distinct species from that in man. Their differential characters may be tabulated as follows:

Criterion	*C. Intestinalis*	*C. davainei*
Size of the tropozoite...	12.2 by 8 μ (Da Fonseca) 13 to 16 μ by 7 to 9 μ	10 to 15 μ by 4 to 7 μ
Cystomal character	The external siderophilous rim has a deep, somewhat acutely pointed undulation mesiad. It takes the stain more heavily than the internal one	The external siderophilous has a wavy and gradual undulation. It is less heavily stained
Cyst	7.9 by 7.2 μ. Top of the cyst wider. Cyst less pyriform	7.5 by 6.5 μ. Top of the cyst more pointed. Cyst distinctly pyriform

SUMMARY

Observations were made on *Chilomastix intestinalis* Kuczinski from two naturally parasitized guinea-pigs and from two artificially infected ones.

The site of infection was determined to be in the lumen of the intestinal tract from about two inches above the cecum posteriorly to the rectum. The tropozoites were found to be cecal, partly intestinal, and partly colonic, lumen-dwelling parasites while the cysts occur at all levels but are more abundant posteriorly and in the fecal pellets. No evidence of tissue invasion could be found. A neuromotor system including the parabasal body and axostyle were described in *C. intestinalis,* similar to those described by Kofoid and Swezy (1920) for *C. davainei.*

No division phase has been encountered in the free forms while undoubted cases of apramitotic fission were seen in a cyst.

Chilomastix intestinalis of the guinea-pig is distinct from *C. davainei* of man. Its cysts have a broader, less constricted anterior region and are stouter and somewhat wider. The external limb of the cytostomal rim of *C. intestinalis* has a deeper, more acutely pointed, lateral constriction than the more gently undulating one of *C. davainei.*

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LEIVA—CHILOMASTIX INTESTINALIS

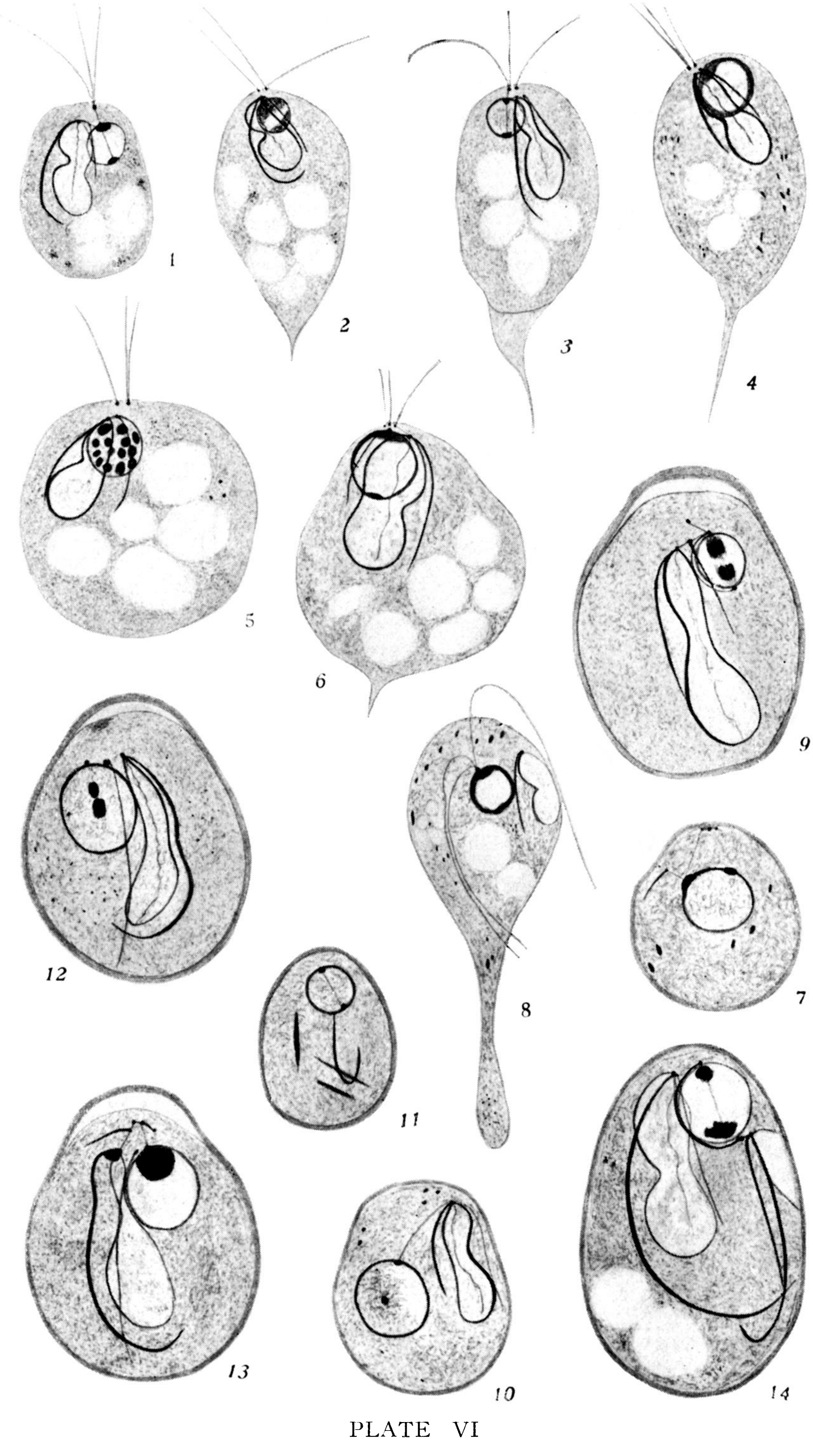

PLATE VI

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Explanation of Plate VI

Chilomastix intestinalis

All figures drawn with camera lucida from smeared preparations fixed in Schaudinn's fluid and stained in Heidenhain's iron-haematoxylin. × 4800, unless otherwise stated.

Fig. 1.—Small form, possibly just emerged from cyst. Trophozoite showing usual disposition of peripheral chromatin massed at opposite poles of nucleus. Note origin of anterior flagella from common blepharoplast. × 3200.

Fig. 2.—Trophozoite showing nucleus with great quantity of massed polar chromatin. × 3200.

Fig. 3.—Trophozoite showing spiral groove about posterior end; caudal area slightly above upper surface of rest of body. × 3200.

Fig. 4.—Free form with exceptionally long caudal appendage. Note peripheral distribution of chromatin on nuclear membrane. × 3200.

Fig. 5.—Rounded-up individual with two of its anterior flagella arising from a common blepharoplast; chromatin in form of small particles scattered throughout nucleus; endoplasm full of big vacuoles. × 3200.

Fig. 6.—Cytostome wide open, three anterior flagella take origin from three separate blepharoplasts. Possibly flattened in preparation. × 3200.

Fig. 7.—Small cyst of *C. intestinalis.* Massed peripheral chromatin in two blocks at the end of the nucleus, extranuclear rhizoplasts connecting nucleus with blepharoplasts; only trace of cytostome; chromatoidal inclusions present as oval, darkly staining bodies.

Fig. 8.—Form with clubbed caudal process produced by metabolic movement of body, or sloughing off of part of cytoplasm. × 3200.

Fig. 9.—Large cyst. Cytostomal margins stand out clearly; chromatin divided as in early anaphase; two centrosomes connected by paradesmose.

Fig. 10.—Encysted form with nucleus possessing central karysome.

Fig. 11.—Cyst with chromatoidal bodies in the form of splinters.

Fig. 12.—Parabasal body well developed. Chromatin appears to be undergoing division.

Fig. 13.—Cyst showing the connection of nucleus with blepharoplasts by rhizoplasts; single large mass of peripheral chromatin at one pole of nucleus.

Fig. 14.—A dividing encysted form. Chromatin mass is parted in anaphase; centrosomes at opposite poles, outside the nucleus, connected by extra-nuclear paradesmose. Both parabasals prominent; axostyle on right very distinct. Note elongated shape of cyst. Only part of other cytostome visible.